Solid Phase Extraction Gas Chromatography/Electron Capture Detector Method for the Determination of Organochlorine Pesticides in Wildlife Whole Blood

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A gas chromatographic method for the analysis of 10 organochlorine pesticides in 0.5 mL of whole blood is described. Sample preparation involved an ethyl ether and hexane extraction, followed by a silica solid phase extraction cleanup. The pesticides are quantified by gas chromatography/electron capture detection. Method limits of detection ranged from 1.1 to 5.2 μg/L. The mean and standard deviation for the recovery of 10 pesticides was 97.9 ± 5.5%. Recoveries from whole blood were comparable to recoveries from plasma. This indicates that the preparation of plasma is unnecessary for the quantification of organochlorine pesticides in blood. This approach is particularly useful as a nonlethal approach for monitoring pesticide contamination in small animals for which the volume of blood is limiting.

Keywords: *Solid phase extraction; dieldrin; organochlorine pesticides; capillary gas chromatography; whole blood; plasma*

INTRODUCTION

The literature on the determination of organochlorine pesticides (OCPs) in human blood indicates that most investigators prepared sera or plasma from whole blood prior to extraction (*1*–*4*). In recent work performed in our laboratory for biomonitoring at the Rocky Mountain Arsenal (RMA), we analyzed numerous wildlife plasma samples for OCPs. The main OCP contaminant on the arsenal was dieldrin, likely due to a pesticide production facility that previously occupied the site. In addition to dieldrin, biomonitoring was performed for aldrin, isodrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, *p,p′*-DDE, endrin, and *p,p′*-DDT.

All pesticide manufacturing operations on the RMA ceased in 1982. In 1987 cleanup of the RMA was initiated. The arsenal is now being remediated for public use and has been designated a National Wildlife Refuge. As part of the remediation and restoration process, biomonitoring of many wildlife species is ongoing. Whenever possible, nonlethal sampling approaches are preferred. In support of this effort, methodology for the quantification of OCPs in wildlife plasma was developed by our laboratory (*5*). Local and migratory birds have become a major issue for monitoring of the RMA. Obtaining adequate blood samples is difficult with some birds; therefore, plasma analysis is not the best approach for long-term biomonitoring of OCPs due to the potentially small sample size. To overcome this sample size obstacle inherent in the plasma method, we developed a method for the analysis of OCPs in 0.500 mL of whole blood stabilized with the anticoagulant heparin. The performance of the new whole blood method with the previous plasma method (*5*) were compared by fortifying whole blood at 50 ppb using a mixed standard of 10 different OCPs and simultaneously extracting whole blood and plasma. The plasma was obtained by centrifuging fortified whole blood, which was then extracted using the plasma method (*5*), whereas whole blood samples were analyzed using the new methodology described in this paper.

MATERIALS AND METHODS

Equipment. A Hewlett-Packard (HP) (Palo Alto, CA) model 5890 series II gas chromatograph equipped with electronic pressure control, dual electron capture detectors, and dual 7673A autosamplers was used to quantify organochlorine pesticides in blood extracts. Solid phase extraction columns (SPE) contained 1 g of silica in a 3-mL reservoir, and Vacmaster sample processing stations were from Jones Chromatography (Lakewood, CO). Gas chromatography (GC) expendables used included inlet liners, silanized glass wool, and gold inlet seals and were from Restek Corp. (Bellefonte, PA).

Chemicals. Organochlorine pesticide standard solutions for lindane, aldrin, isodrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, dieldrin, *p,p′*-DDE, endrin, and *p,p′*-DDT were obtained from both Chem Service Inc. (West Chester, PA) and Supelco (Bellefonte, PA). Ether, anhydrous 99+%, was from Aldrich Chemical Co. (Milwaukee, WI). Pesticide residue grade hexane was from Burdick & Jackson (Muskegon, MI). Acetone was from Fisher Scientific (Pittsburgh, PA). Control chicken whole blood stabilize with heparin was obtained from Animal Technologies, Inc. (Tyler, TX).

Standard Preparation. A mixed standard was prepared by combining aliquots of each standard solution and diluting with acetone (10.0 μg/mL). This was diluted in acetone to prepare standard solutions for fortification (1.00 and 0.500 μg/mL). Instrument calibration standards and diluted standard solution were prepared in hexane.

Sample Fortification. Control blood was fortified with a mixed standard containing lindane, aldrin, isodrin, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, *p,p′*-DDE, dieldrin, endrin, and *p,p′*-DDT. For method validation, control blood was fortified at six levels: 25, 50, 100, 250, and 500 μg/L of each compound; the blank was fortified at 250 μg/L with only the surrogate (lindane). Lindane was chosen as a surrogate because of its similarities to the analytes of interest and there was no prior use of lindane at this site.

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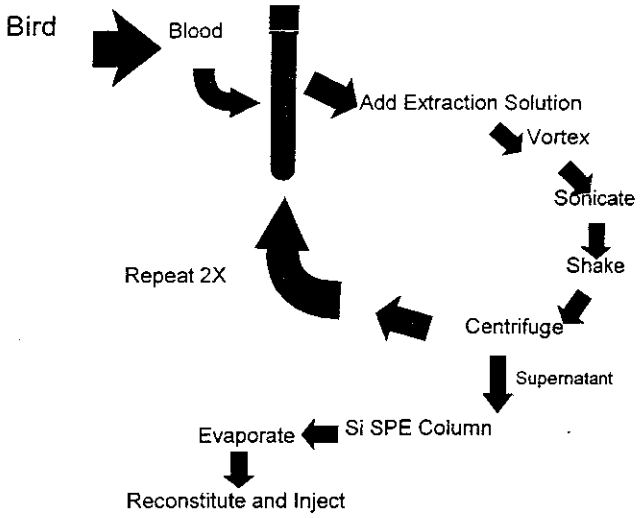


Figure 1. Flow chart of procedure.

Sample Preparation. As summarized in Figure 1, a 0.500-mL aliquot of homogeneous blood was transferred to a 10-mL glass centrifuge tube, fortified, and allowed to equilibrate for 1 h. The analytes were extracted from the blood using two 3-mL aliquots of ethyl ether followed by one 3-mL aliquot of 50:50 hexane/ether, vortex mixed, sonicated for 10 min, hand shaken for 1 min, and centrifuged for 2 min (≈1400*g*). The extract was carefully transferred to a silica SPE and loaded onto the sorbent with a low vacuum (−0.05 to −0.1 bar). The pipet used for transferring the extract was rinsed onto the sorbent with 50:50 hexane/ether. To ensure all of the analytes of concern are not retained, the sorbent is rinsed with 4 mL of 50:50 hexane/ether. The entire eluate for each sample was collected in a clean 25-mL evaporation tube, which had been calibrated to a 0.500-mL volume with hexane. The final amount of solvent in the column was removed under a gentle vacuum (−0.2 bar). The total volume of extract should be ~14 mL. The extracts were concentrated to <0.500 mL under a gentle stream of nitrogen in a fume hood, equilibrated to room temperature, and brought to a final volume of 0.500 mL with hexane. The samples were then capped, vortex mixed, and transferred to GC vials for quantification of OCPs via GC analysis.

Gas Chromatography. The inlet temperature was 250 °C, and the detector temperature was 350 °C. The GC parameters were controlled utilizing HP ChemStation software and an HP Vectra XM series 3 computer. The carrier gas was helium (3 cm/s), and the makeup gas was argon/methane (60 mL/min). The quantitation column was a 30 m × 0.25 mm i.d. fused silica, HP-5 cross-linked 5% phenyl methyl silicone stationary phase, 0.25-μm film thickness (Hewlett-Packard). The confirmation column was a 30 m × 0.25 mm i.d. fused silica DB-17 cross-linked 50% phenyl methyl siloxane stationary phase, 0.15 μm film thickness (J&W Scientific, Folsom, CA).

The oven temperature program for quantitation and confirmation was as follows: 50 °C for 0.25 min, 60 °C/min to 100 °C, hold for 0 min, 15 °C/min to 190 °C, hold for 2 min, 10 °C/min to 230 °C, hold for 11 min, 60 °C/min to 300 °C, hold for 8.58 min. The electronic pressure program for the quantitation column maintained the pressure at 16 psi; the confirmation column inlet pressure was held at 80 psi for 2 min, and then 16 psi for the remainder of the run. A double-tapered 4 mm i.d. liner was used for the quantitation column, whereas a single-tapered 4 mm i.d. inlet liner packed with deactivated glass wool was used on the confirmation column. Both columns had a 1-μL injection volume.

Method Validation. Detector linearity was determined by linear regression analysis of five-point calibration curves (response versus concentration) for each analyte. After $r^2 \geq 0.99$ had been achieved, linear regression equations were used to quantify analytes in samples. Fortified blood samples (six levels including a blank) were prepared using the above procedure and analyzed by GC, and percent recoveries were determined for each analyte at each fortification level on two consecutive days (*6*). Method limits of detection (MLODs) were calculated from the 25 μg/L fortified blood and control chromatograms. MLODs were calculated as the quantity of analyte required to give a response of 3 times the baseline noise at the expected retention time of the analyte in the chromatogram of a nonfortified blood extract.

Quality Control. To ensure consistent instrument performance, prior to the GC analysis of any samples, a standard containing 100 μg/L endrin and 200 μg/L DDT was analyzed. For analysis to proceed, degradation was determined to be ≤20% for each compound and ≤30% for both compounds. Also, a 250 μg/L instrument calibration check standard was analyzed at the beginning, after every 10 samples, and at the end of each analytical run. The magnitude of response for all 10 analytes was required to match the response of the 250 μg/L standard in the calibration curve ±25%. Additionally, retention matches of ±0.05 min were required.

Surrogate recoveries were used to monitor individual sample extraction efficiency and instrument performance. In addition to analysis on the quantitation column, extracts of all samples found to contain OCPs were confirmed by GC analysis on the confirmation column.

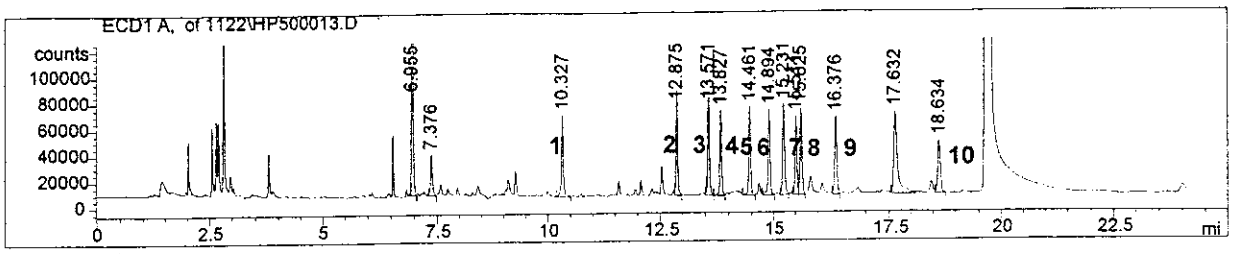
RESULTS AND DISCUSSION

Chromatography. Due to the commercial unavailability of wildlife blood and plasma, chicken blood stabilized with the anticoagulant heparin was utilized for method development, validation, and quality control samples. Control chicken blood proved to be acceptable for these purposes as indicated by the lack of chromatographic responses at the retention times for the analytes of interest (Figure 2). Although the analysis of whole blood extracts produced more chromatographic peaks than plasma in the region of interest, none of the peaks interfered with any of the analytes of interest.

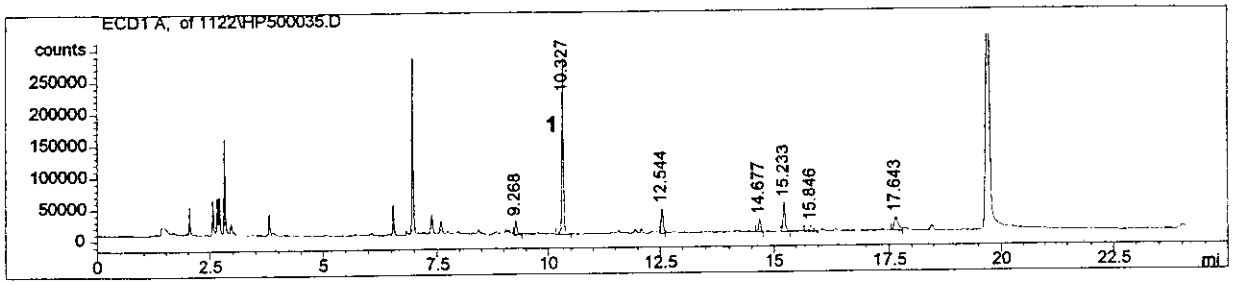
Method Validation. The results of the method validation experiments are presented in Table 1. For dieldrin, the analyte of primary concern, the mean recovery was 98.6% and the standard deviation was 5.7%. Mean recoveries of the other analytes of primary concern, aldrin, endrin, *p,p′*-DDT, and *p,p′*-DDE, were 86.5 ± 10, 106 ± 3.1, 104 ± 12, and 96.0 ± 8.9%, respectively. Mean recovery of lindane, the compound added to all samples as a surrogate, was 97.0 ± 4.3%. This is similar to the 97.9 ± 9.4% mean recovery for all compounds, indicating the suitability of lindane as a surrogate standard for these analyses. MLODs as calculated during method validation are presented in Table 2. The MLOD for dieldrin was 3.1 μg/L. The MLODs for the other analytes of primary concern ranged from 1.1 μg/L for *p,p′*-DDT to 5.2 μg/L for endrin.

Comparison of Blood versus Plasma Methodology. Ten aliquots of 0.500 mL of chicken whole blood with heparin were fortified at 50 μg/L with the 10 compounds of concern. Two more aliquots to be used as controls were fortified with only the surrogate (lindane) at 250 μg/L. Of the 12 whole blood samples fortified, 6 were extracted using the methodology for whole blood described in this paper. Six were centrifuged, and the resulting plasma was extracted using a previously published method (*5*). To summarize, plasma samples were transferred to a vacuum manifold containing conditioned C2 over C18 SPE columns in series. Three quality control plasma samples were added at this point. They were fortified at 50 μg/L and then extracted and assayed with the plasma samples to ensure the methodology was adequate because the sample size was

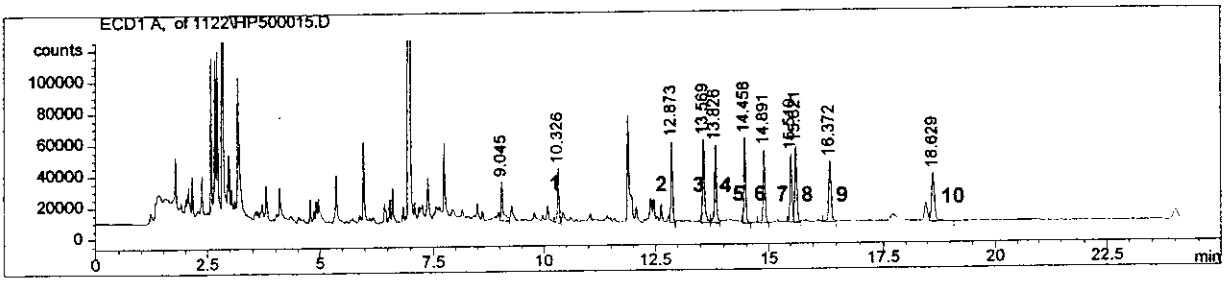
50 μg/L Fortified Chicken Whole Blood:



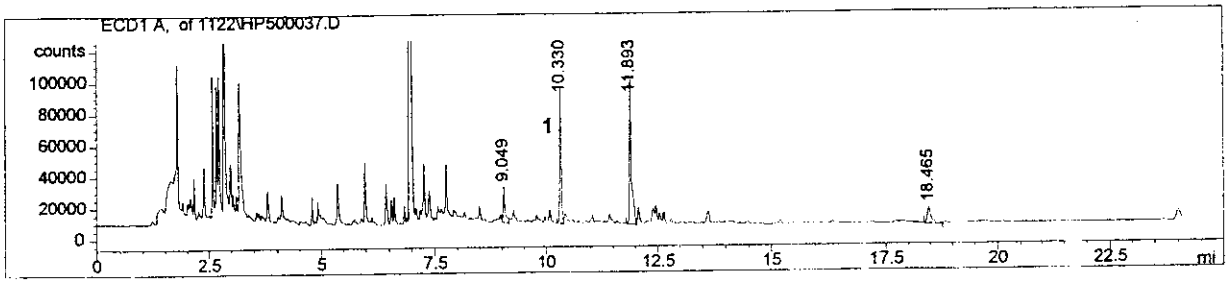
Control Chicken Blood:



50 μg/L Fortified Chicken Plasma:



Control Chicken Plasma:



(1) lindane (surrogate), **(2)** aldrin, **(3)** isodrin, **(4)** heptachlor epoxide, **(5)** *trans*-chlordane, **(6)** *cis*-chlordane, **(7)** p,p'-DDE, **(8)** dieldrin, **(9)** endrin and **(10)** p,p'-DDT.

Figure 2. Chromatograms.

changed from 1.000 to 0.500 mL. One milliliter of formic acid was added to each sample, which was then loaded onto the SPEs. The columns were allowed to dry under vacuum. A silica SPE was added below the C2 and C18 columns for sample cleanup, and the analytes were eluted with four 3-mL aliquots of 1:1 hexane/ether. Extracts were evaporated to <0.500 mL and then reconstituted with hexane to 0.500 mL. All samples were analyzed with the instrument parameters from the whole blood method.

The data presented in Figure 3 show that the analyte recoveries from whole blood were comparable to those observed from plasma. Four compounds, lindane, aldrin, *cis*-chlordane, and endrin, have better recoveries in whole blood. Least-squares means of the three sets of samples were graphed to make comparisons. Pairwise comparisons of least-squares means were performed using the Bonferroni method (7). Results indicated there were significant differences in blood and plasma recoveries for lindane, aldrin, *cis*-chlordane, and endrin. The

Table 1. Method Validation Mean Percent Recoveries[a]

compound	25 μg/L	50 μg/L	100 μg/L	250 μg/L	500 μg/L	grand mean	SD
lindane	90.2	100	99.5	99.8	95.4	97.0	4.3
aldrin	68.9	89.2	90.7	94.1	89.4	86.5	10
isodrin	79.5	97.4	99.5	101	96.3	94.8	8.7
heptachlor epoxide	82.0	96.1	103	101	95.5	95.5	8.2
trans-chlordane	84.5	100	104	105	99.4	98.5	8.1
cis-chlordane	80.9	104	109	108	104	101	12
p,p'-DDE	80.9	96.2	101	104	98.5	96.0	8.9
dieldrin	89.8	97.9	104	104	98.0	98.6	5.7
endrin	107	103	110	109	103	106	3.1
p,p'-DDT	86.2	98.5	111	115	110	104	12

[a] Mean percent recoveries were calculated from quantitative and confirmation columns on two separate days ($n = 4$). The total mean recovery for all compounds equals 97.9 ± 9.4% ($n = 50$).

Table 2. Method Limits of Detection in Whole Blood

compound	av MLOD (μg/L)
lindane	not calcd—surrogate spiked in control samples
aldrin	4.2
isodrin	2.1
heptachlor epoxide	1.4
trans-chlordane	2.3
cis-chlordane	4.2
p,p'-DDE	2.1
dieldrin	3.1
endrin	5.2
p,p'-DDT	1.1

[a] MLODs were calculated using a control and a whole blood sample fortified at 25 μg/L, assayed on two separate days on the quantitation column ($n = 2$).

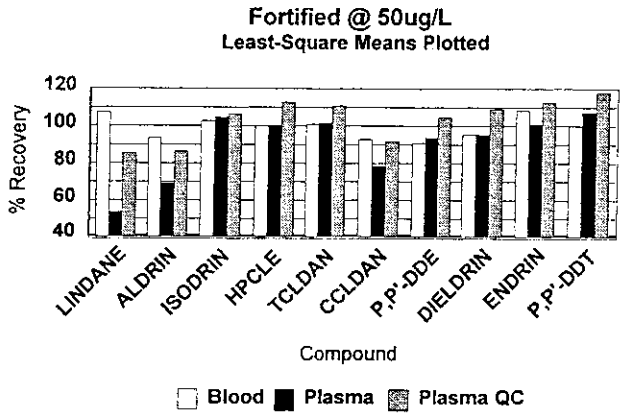


Figure 3. Percent recovery for whole blood and plasma methodologies.

blood method was more efficient in recovering these compounds, possibly due to analyte loss when the plasma was separated from blood cells. There were also significant differences in plasma and plasma quality control recoveries for lindane, aldrin, and *cis*-chlordane. This further supports the assumption that some analytes may be lost when the plasma is separated.

Table 3 shows MLODs for the two methods. The MLOD for plasma was higher than for whole blood in every case but endrin. This comparison suggests that the blood method was generally more sensitive than the plasma method. However, an analysis of variance indicated a strong relationship between compound and matrix interactions. This suggests that the MLOD depends on the matrix and compound. As indicated in Table 3, the MLOD for isodrin in blood is 3.0 mg/L, whereas that for plasma is 22 mg/L; however, that for heptachlor epoxide in blood is 2.6 mg/L, whereas in plasma it is 2.7 mg/L.

Table 3. Method Limits of Detection in Chicken Whole Blood and Plasma[a]

	MLOD (μg/L)	
compound	whole blood	plasma
lindane	not calcd—surrogate spiked in control samples	
aldrin	4.1	7.6
isodrin	3.0	22
heptachlor epoxide	2.6	2.7
trans-chlordane	2.5	4.8
cis-chlordane	1.8	2.8
p,p'-DDE	1.4	4.6
dieldrin	2.1	2.7
endrin	9.6	4.9
p,p'-DDT	2.1	4.1

[a] MLODs were calculated using a control and samples fortified at 50 μg/L ($n = 5$).

The data presented here suggest that the preparation of plasma prior to analysis for quantification of OCPs is unnecessary and may produce some analyte partitioning, which can influence the amount of OCP observed in a sample. The whole blood methodology permits collection of smaller blood volumes than is required for plasma analysis and is more amenable to smaller species (for which the quantity of blood is limited) and is therefore more broadly applicable to a wider range of wildlife species. This approach could be applied to the analysis of other pesticides, drugs, and organic contaminants in wildlife.

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